

# Human biting rhythm of *Anopheles gambiae* s.l. and sleeping behaviour of pregnant women in a lagoon area in Southern Benin

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## Research note

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# Abstract

## Objective

In the framework of EVALMOUS study aiming to assess the use and effectiveness of mosquito nets by pregnant women and other members of their household in a lagoon area in southern Benin, the behaviour of pregnant women relative to the time they go to bed using the net were recorded. Malaria vectors biting rhythm, *Plasmodium falciparum* infection and insecticide resistance genes in malaria vectors were also determined.

## Results

Overall, 3848 females of *Anopheles gambiae s. l.* were collected and 280 pregnant women responded to the survey. Almost all *Anopheles gambiae s. l.* tested were *Anopheles coluzzi* with the exception of two, which were *Anopheles melas*. The CSP index in malaria vector was 1.85% and the allelic frequency of *kn* gene was 74.4%. Around 90% of bites and *Plasmodium falciparum* transmission occurred between 10 p.m. and 6 a.m., which coincides with the period when more than 80% of pregnant women were under bednet. Despite a slight early evening and early morning biting activity of malaria vectors in the study area, the good use of nets might remain a useful protection tool against mosquito biting and malaria transmission.

## Introduction

Since 2000, a significant expansion of financial support and scale-up of malaria control tools were observed [1]. Progress in terms of reduction in malaria cases and deaths followed this scale-up of malaria control tools. Despite this, malaria remains a major public health concern in Africa, with an estimated 213 million cases and 380 000 deaths in 2018 [2]. In Benin, malaria cases increased from 1 044 235 and 1869 deaths to 1 768 450 malaria cases and 2138 deaths between 2014 and 2018.

In many malaria endemic areas including Benin, Long Lasting Insecticidal treated Nets (LLIN) and Indoor Residual Spraying (IRS) represent the main interventions for malaria vector control [2] as recommended by World Health Organisation (WHO). These indoor insecticide-based methods depend on various factors among which vectors behaviour (such as human biting rhythm and endo / exophagy), human behaviour (e.g. sleeping behaviour) and insecticide resistance mechanisms in vectors. Insecticide resistance is well documented in malaria vectors in malaria endemic countries [3, 4]. Furthermore, numerous studies across endemic countries reported behavioural changes of malaria vectors [5–7]. Early morning biting activity of *Anopheles funestus* following the universal coverage of LLIN was reported in Southern Benin and authors stated that these findings might have direct consequences for malaria control in Africa [5]. EVALMOUS study assessed the use and the effectiveness of mosquito nets by pregnant women and other members of their household in preventing malaria [8]. This study was carried out in Sô-Ava district, a lagoon area with high risk for malaria vectors proliferation [9]. Entomological surveys and questionnaire were carried out to assess malaria vectors human biting activity and the sleeping behaviour of pregnant women.

## Methods

### Study area

EVALMOUS study was carried out in 15 villages of Sô-Ava district (6° 28' 00" N 2° 25' 00" E) located in Nokoue Lake in Southern Benin. Sô-Ava is one of the eight districts of Atlantic department with about 100 000 inhabitants and a surface area of 218 km<sup>2</sup>.

### Mosquito collection

Mosquito collection was carried out in all villages selected for EVALMOUS study, using human landing catches method (HLCs) as described by Djènontin *et al*, 2010 [10]. Mosquito collection was carried out both indoors and outdoors in three sites per village for one night during the rainy season between July and August 2016. Mosquitos were collected from 7 p.m. to 9 a.m. Collector teams were rotated among the collection points to minimize sampling bias.

### Laboratory analysis

Mosquitoes were identified to species level using morphological criteria according to the identification keys [11, 12]. All mosquitoes identified as *Anopheles gambiaesensu lato* (*s. l.*) were stored in individual tubes with silica gel and preserved at -20°C in the laboratory.

Heads and thoraces of anopheline females were tested by enzyme-linked immunosorbent assays (ELISA) for detection of *Plasmodium falciparum* circumsporozoite protein (CSP), as previously described [13].

Genomic DNA was extracted from the abdomen of all mosquito using the extraction buffer Livak [14]. The species was then identified by diagnostic Polymerase Chain Reaction (PCR) using the Scott protocol [15] and molecular characterization according to Santolamazza protocol [16, 17]. The knockdown resistance mutation (*kdr-west*, L1014F) was detected by Taqman allelic discrimination assays as described by Bass *et al* [18].

### Pregnant women sleeping behaviour and bed net use

Data regarding the time at which each pregnant woman entered and exited their house, the time each bed net user entered and exited their sleeping space the night preceding the survey were collected by questionnaire.

### Data analysis

Every hour from 7 p.m. to 9 a.m, the proportion of malaria vectors collected indoors was determined, as well as the proportion of pregnant women under bed nets at each hour from 7 p.m. to 9 a.m the night

preceding the survey.

The mosquito collection period was divided into 3 different timeframes (7 p.m. – 10 p.m.; 10 p.m. – 6 a.m.; 6 a.m. – 9 a.m.). The proportion of mosquito collected at each timeframe (aggressiveness) was calculated, as well as the 95% confidence interval.

CSP index in malaria vectors *Anopheles gambiaes.l.* in each village was calculated as the number of positive vector divided by the total tested. Endophagy was calculated as the number of vectors collected indoors divided by the total collected. The 95% confidence interval of the CSP index and endophagy were also estimated. Allelic frequency of *kdr* gene in *Anopheles gambiaes.l.* at each period was calculated and compared using Chi-square tests.

## Results

### Human biting rhythm and pregnant women sleeping behaviour

Overall, 3848 females of *Anopheles gambiaes. l.* were collected in the 15 villages (Table S1). A high spatial heterogeneity of abundance malaria vectors was observed (figure 1). The number of females of *Anopheles gambiaes.l.* collected ranged from 14 (corresponding to 2.33 bites per person per night in Awomey-Gbèkpa village) to 1114 (corresponding to 185.66 bites per person per night in Todo village). Respectively 1.3%, 90.7% and 8.0% of mosquito's aggressiveness occurred during the timeframes 7 p.m. – 10 p.m.; 10 p.m. – 6 a.m. and 6 a.m. – 9 a.m. (Table S2).

A total of 280 pregnant women responded to the survey. The proportion of pregnant women under bed net from early evening to early morning ranged from around 1% (8 a.m. - 9 a.m.) to 100% (12 a.m. - 3 a.m.) (Figure 2). From 10 p.m. to 6 a.m., where around 90% of aggressiveness and transmission occurred in the study area during the survey, 85.5% of pregnant women were under bed net (Figure 2).

### Malaria vectors species and *Plasmodium falciparum* infection

From the 3848 females of *Anopheles gambiaes. l.* collected, 1383 were selected and successfully identified at species level using PCR. Almost all *Anopheles gambiaes. l.* tested were *Anophelescoluzzi* except 2 which were *Anopheles melas*.

The overall CSP index was  $1.85 \pm 0.01\%$  (Table 1). CSP antigen index showed a spatial heterogeneity ranging from 0% (in Awomey-Gbèkpa, Awomey-Gblon and Nonhouéto villages) to  $5.77 \pm 0.88\%$  in Hounhouè village. Respectively 1.41%, 94.37% and 4.23% of *Plasmodium falciparum* transmission occurred during the periods 7 p.m. – 10 p.m.; 10 p.m. – 6 a.m. and 6 a.m. – 9 a.m. (Table 1).

The endophagy, was estimated on the total sample at  $56.4 \pm 0.01\%$ . Endophagy was significantly higher at the early evening (7 p.m. - 10 p.m.  $66.7 \pm 12.9\%$ ) compared to the early morning (6 a.m. – 9 a.m.,  $45.2 \pm 5.6\%$ ) (Table S2).

## Allelic frequency of *kdr* gene in *Anopheles gambiae s.l.*

The allelic frequency of *kdr* gene in *Anopheles gambiae s.l.* collected in the villages was 74.4%. No significant difference was observed between the allelic frequency of this gene indoors (75.4%) and outdoors (73.3%) on the total sample (Table S3). However, the allelic frequency of *kdr* gene in *Anopheles gambiae s.l.* biting human indoors early morning (6 a.m. – 9 a.m.), 82.0 [77.5-86.5] % seems higher than that of *Anopheles gambiae s.l.* biting human outdoors during the same period (72.8 [68.0-77.5] %).

## Discussion

The present study shows that despite a slight early evening and early morning biting activity of malaria vectors, more than 90% of malaria vectors bites and *Plasmodium falciparum* transmission occurred between 10 p.m. and 6 a.m., a period at which over 80% of pregnant women were under bed net in the study area. The main malaria vector in the study area was *Anopheles coluzzi* with a CSP index and an allelic frequency of *kdr* gene equal respectively to 1.9 % and 74.4%.

The early evening and early morning biting activity reported in this study are consistent with recent findings on *Anopheles funestus* in Southern Benin [5]. Despite this unusual malaria vectors biting activity in the study area, bed net remains a useful protection tool against mosquito biting since more than 90% of bites and *Plasmodium falciparum* transmission occurred between 10 p.m. and 6 a.m., period during which most pregnant women were in bed under bed net. Analysing data collected in 2011 on behavioral interactions between *Anopheles funestus* and humans in two villages of southern Benin, Moiroux et al showed that the true average personal protection of LLINs remained very high (> 80%), despite the change in this vector biting peak [19]. The critical challenge for malaria control in the study area seems to remain the good use, bio efficacy and the physical integrity of LLIN. Indeed, as reported by Hounkonnou et al, around 90% of pregnant women in the study area used bed net, but less than 10% of bed net used by pregnant women attained WHO bio-efficacy criteria [8]. It is also important to note that in the present study, aggressiveness and transmission had a very high spatial heterogeneity ranging from 2.33 bites (0 infected bite) per person per night in Awomey-Gbèkpa village to 185.66 bites (4 infected bites) per person per night in Todo village. These findings highlight the existence of local foci of transmission in the study area where the aggressiveness and transmission remain high even in the timeframe 6 a.m.-9 a.m. when majority of pregnant women were not under LLINs. In Todo village, aggressiveness and transmission were respectively 14.85 bites per person and 0.32 infected bites per person during this timeframe).

The study area is a natural lake influenced by the Atlantic Ocean and previous studies carried out 2 decades ago in neighbouring lagoon areas reported that *Anopheles melas* represents 80% of *Anopheles gambiae s.l.* [20, 21]. The low *Anopheles melas* proportion reported in the present study was recently reported in the same district [9], and could be due to its urbanized nature which has probably led to the proliferation of breeding sites of *Anopheles coluzzii* at the detriment of *Anopheles melas* [9, 20]. Since *Anopheles melas* is known to have a sporozoitic index significantly lower than that of *Anopheles coluzzii* [20–22], the relative proliferation of breeding sites of *Anopheles coluzzii* is a potential health concern

considering malaria transmission in this area. However, subsequent increase in malaria transmission in this area have been masked by the large scale implementation of vectors control tools (e.g. Long Lasting Insecticidal Nets) [9]. A recent study carried out in this study area showed a general high possession of bed nets by the pregnant women with more than 8 out of 10 women using it [8].

The allelic frequency of *kdr* gene in *Anopheles coluzzii* reported in the present study is consistent with that reported recently in neighbouring villages [9] and was lower than 100% observed in Cotonou, a neighbouring district. As fishing is the most important economic activity in the study area, there is a lower use of insecticides linked to agriculture. The insecticide resistance selection in this area could be due to the domestic use of insecticide for vectors control [9]. In addition, an increase in the activity of vegetable farming in neighbouring districts [23] has led to the use of insecticides in an improper manner to control vegetable pests and pesticide residues from these areas can also contribute to insecticide resistance selection in the study area [9].

## Conclusion

The present study revealed a non-negligible early evening and early morning biting activity of malaria vectors in the study area. However, the major part of transmission occurs at a period when more than 80% of pregnant women were on bed under bed net, indicating that the good use of LLIN remains a useful protection tool against mosquito biting and malaria transmission.

## Limitations

This study design is cross-sectional and mosquito collection was carried out in 3 different points only for one night due to logistic reasons. This could be considered as a limitation, and a longitudinal design may have brought a higher level of evidence. However, the data collected is precious because such data; aggressiveness rhythm and human behaviour collected simultaneously in the same setting, are rare. Essentially, it is of crucial importance to monitor these indicators at a high scale and with a higher frequency.

## Abbreviations

**CSP:** Circumsporozoite Protein

**ELISA:** Enzyme-Linked Immunosorbent Assays

**HLCs:** Human Landing Catches

**IRS:** Indoor Residual Spraying

***Kdr*:** Knockdown Resistance

**LLIN:** Long Lasting Insecticidal treated Nets

**PCR:** Polymerase Chain Reaction

*S. l.: sensu lato*

**WHO:** World Health Organisation

## **Declarations**

# **Ethics approval and consent to participate**

Ethical clearance was given for the study by the National Ethical Committee in Benin (Comité National d’Ethique pour la Recherche en Santé). Collectors gave prior written informed consent and were vaccinated against yellow fever and medically supervised by local physicians in case of illness.

## **Consent for publication**

Not applicable

## **Availability of data and material**

The datasets during and/or analysed during the current study are available from the corresponding author on reasonable request.

## **Competing interests**

The authors declare that they have no competing interests

## **Funding**

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## **Author’s contributions**

AD and GC conceived of the study. AD and AB, CS have participated in the data collection on field. AB and CS carried out laboratory assays. AD and GC have participated in the analysis and interpretation of data. SE has realized the map of the study area. The manuscript was drafted by AD. SE revised the language. All authors read and approved the final manuscript.

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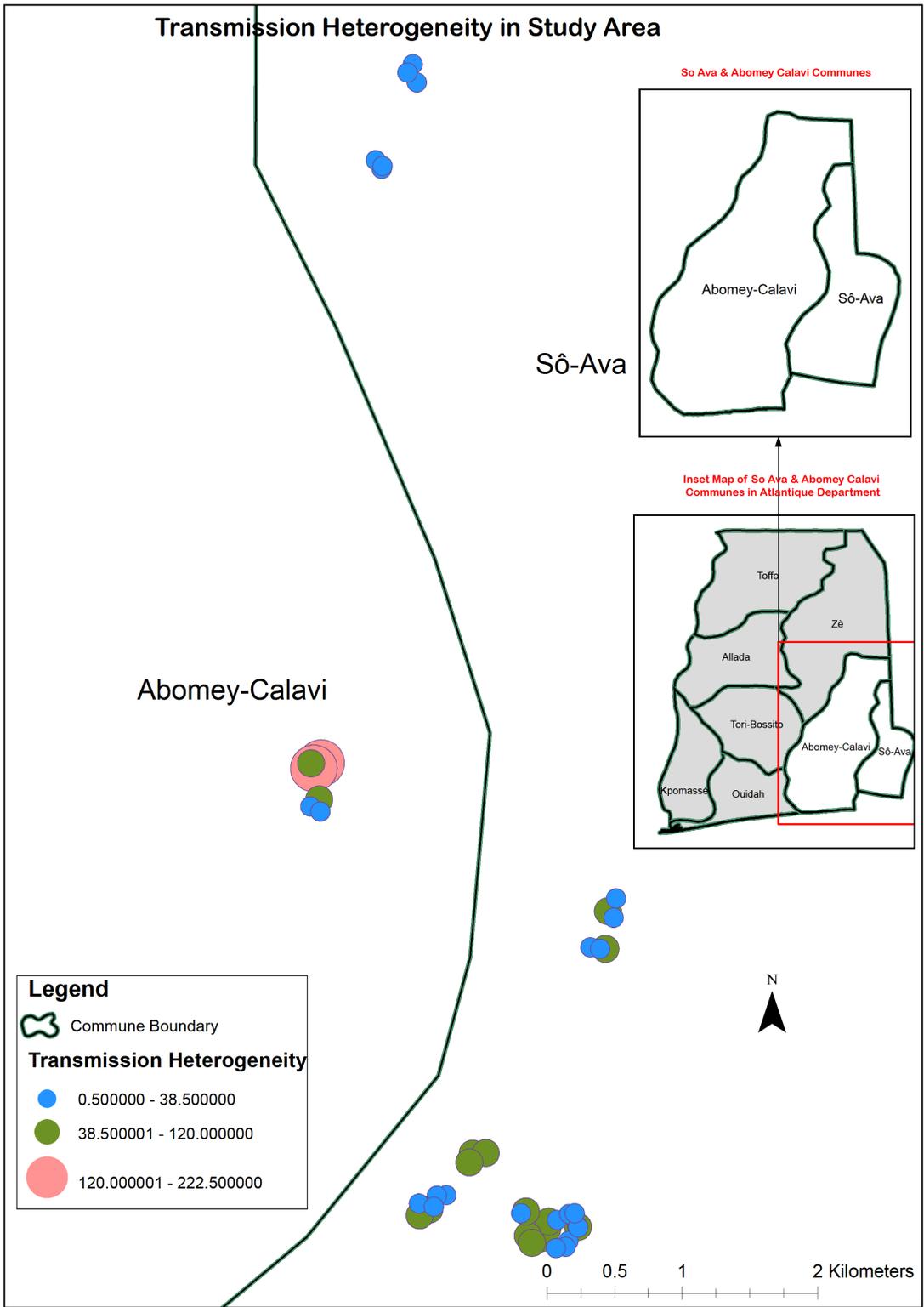
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## Tables

**Table 1:** CSP antigen index in malaria vectors and *Plasmodium falciparum* transmission both indoor and outdoor per period with 95% CI

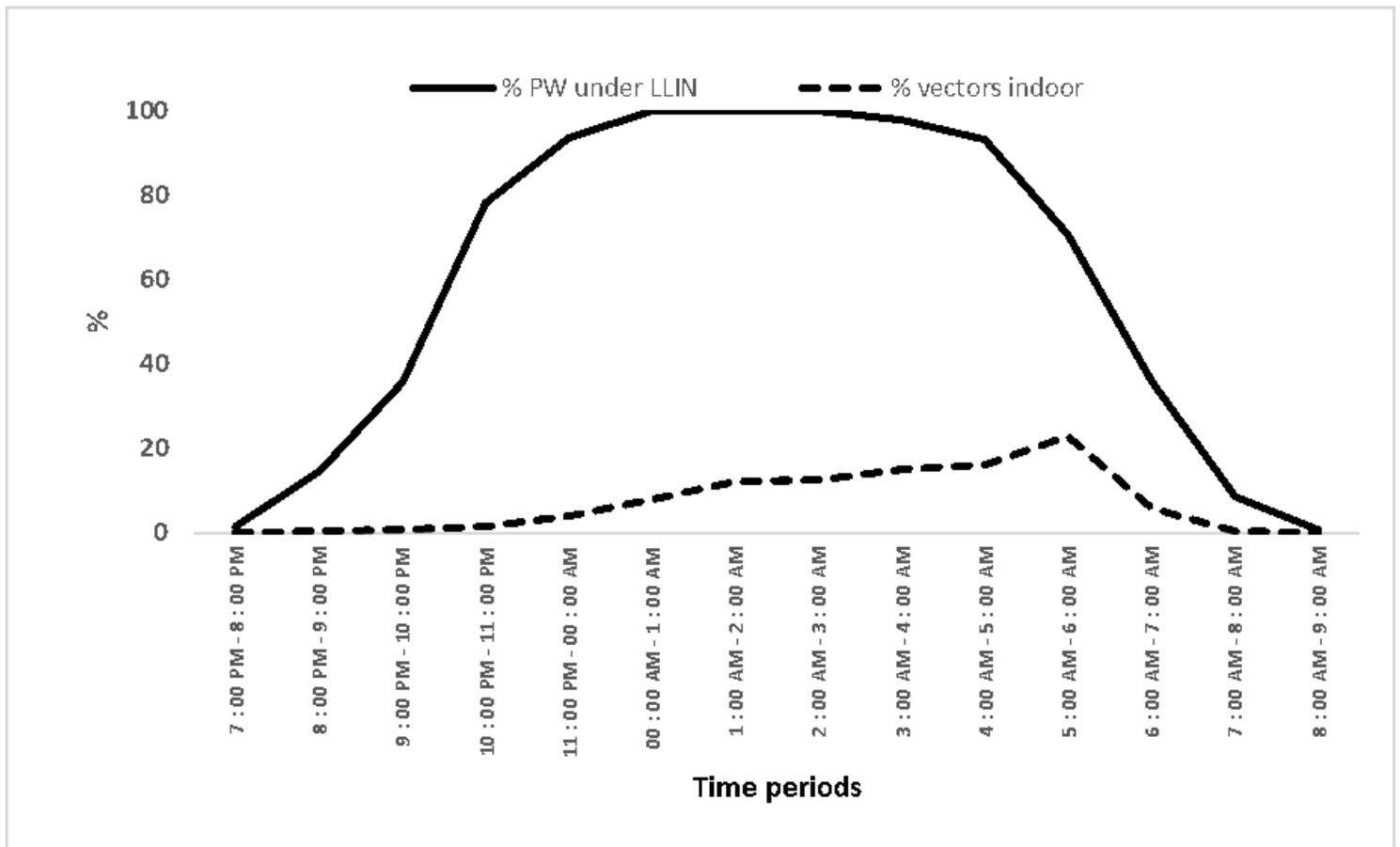
|                  |         | N vectors tested | N vectors CSP <sup>+</sup> | CSP <sup>+</sup> rate (%) | 95%CI (%) | Transmission |
|------------------|---------|------------------|----------------------------|---------------------------|-----------|--------------|
| 7 p.m. - 10 p.m. | Indoor  | 34               | 1                          | 2.94                      | 0.97      | 1.41         |
|                  | Outdoor | 17               | 0                          | 0.00                      | 0.00      | 0.00         |
|                  | Total   | 51               | 1                          | 1.96                      | 0.53      | 1.41         |
| 10 p.m. - 6 a.m. | Indoor  | 1998             | 39                         | 1.95                      | 0.01      | 54.93        |
|                  | Outdoor | 1492             | 28                         | 1.88                      | 0.02      | 39.44        |
|                  | Total   | 3490             | 67                         | 1.92                      | 0.01      | 94.37        |
| 6 a.m. - 9 a.m.  | Indoor  | 139              | 1                          | 0.72                      | 0.12      | 1.41         |
|                  | Outdoor | 168              | 2                          | 1.19                      | 0.13      | 2.82         |
|                  | Total   | 307              | 3                          | 0.98                      | 0.06      | 4.23         |
| Total            | Indoor  | 2171             | 41                         | 1.89                      | 0.01      | 57.75        |
|                  | Outdoor | 1677             | 30                         | 1.79                      | 0.02      | 42.25        |
|                  | Total   | 3848             | 71                         | 1.85                      | 0.01      | 100          |

## Figures



**Figure 1**

Spatial heterogeneity of malaria vectors abundance in the study area



**Figure 2**

Proportion of pregnant women (PW) under bed nets and proportion of vectors collected indoor per period

## Supplementary Files

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